MEDIZINISCHE UNIVERSITÄT
INNSBRUCK

Programme and Abstract book

2nd MUI-START Symposium

PROGRAMME

June 28 th, 2013; Lecture Hall Pharmacology, Peter-Mayr-Straße 1-1a
11:30 – 14:00

TIME	TITLE	Abstract
11:30 – 11:40	**Welcome Address VR Prof. Günther Sperk**	
	Oral presentations	
11:40 – 11:50	**Dr. rer.nat. Galina Apostolova** - "Neuronal activity and BDNF drive the expression of nuclear architecture protein Satb2 in primary hippocampal neurons"	O 01
11:50 – 12:00	**Mag.rer.nat. Dr. phil. Martin Puhr** - "EMT- A possible loop hole for cancer cell survival during docetaxel chemotherapy and basis for a highly metastatic cancer cell phenotype"	O 02
12:00 – 12:10	**Dr. med.univ. Rupert Oberhuber** - "Tetrahydrobiopterin Preconditioning Saves Murine Pancreatic Isografts from Brain Death Exacerbated Ischemia Reperfusion Injury"	O 03
12:10 – 12:20	**Mag.rer.nat. Ulrike Binder PhD** - *"Galleria mellonella* as a host model to study invasive fungal infections due to *Mucorales*"	O 04
12:20 – 12:30	**Dr. phil. nat. Janine Kimpel** - "The Viral Vector Vaccine VSV-GP Boosts Immune Response upon Repeated Applications"	O 05
12:30 – 12:40	**Priv. Doz. Dr. Raimund Helbok** - "Pathophysiology of Early Brain Injury in Aneurysmal Subarachnoid Hemorrhage Patients - A Microdialysis Study"	O 06
12:40 – 13:00	***Coffee break***	

TIME	*Poster introductions*	Abstract
13:00 – 13:01	**Dr. Birgit Frauscher** - "Minor motor phenomena during sleep: A quantitative study in a representative sample of healthy sleepers"	P 01
13:01 – 13:02	**Dr.med.univ. Peter Lackner** - "NMDA-receptor mediated excitotoxicity is involved in the pathogenesis of experimental cerebral malaria"	P 02
13:02 – 13:03	**Dr.med.univ. Markus Theurl** - "Therapeutic Angiogenesis By The Neuropeptide Catestatin"	P 03
13:03 – 13:04	**Dr.med.univ. Theresa Hautz** - "Treatment with anti-IL-1β prolongs limb allograft survival in an experimental model of vascularized composite allotransplantation"	P 04
13:04 – 13:05	**Dr.med.univ. Martina Stichlberger** - "Effects of Vasopressin on Migration and Oxygen Free Radical Release of Human Leukocytes"	P 05
13:05 – 13:06	**Mag. Selma Tuzlak** - "Impact of the prosurvival Bcl-2 family member A1 on T cell immunity"	P 06
13:06 – 13:07	**MD PhD Manfred Nairz** - "The Erythropoietin-Analogue ARA290 Ameliorates the Course of Experimental Colitis"	P 07
13:07 – 13:08	**Mag.rer.nat. Michael Blatzer PhD** - "In Aspergillus fumigatus, BolA-deficiency causes growth defects dependent on temperature, oxidative stress as well as supply of iron and oxygen"	P 08
13:08 – 13:09	**DI (FH) Dr.rer.nat. Judith Hagenbuchner** - "Regulation of glycolysis and mitochondrial respiration by BIRC5/Survivin in neuronal tumor cells"	P 09
13:09 – 13:10	**Ass.Prof. Dr. med.univ. Joachim Schmutzhard** - "Die Mitbeteiligung des Innenohrs bei der Sepsis im CLP Maus Model"	P 10
13:10 – 14:00	***Coffee and Poster discussion***	

Oral Talks

Oral presentation should last no more than 5 min to have 5 min for discussion. Please provide the powerpoint presentation of your talk at 11:00 in the lecture hall stored on an USB stick.

Poster Presentations

The size of posters should be A0 = 80 cm HORIZONTAL x 120 cm VERTICAL.

The Poster should include: title, abstract, material and methods, results and discussion.

Posters can be mounted previously to the symposium on the poster walls in the seminar rooms. Please mount the posters accordingly to the numbers in the programme and which will be also posted on the poster walls.

The posters should be first introduced by the project leader within 1 minute with the help of one or two overview slides (powerpoint) in the lecture hall.
Please provide the powerpoint slides at 11:00 in the lecture hall stored on an USB stick.

The aim of this 1-minute-introduction should be to draw interest to your poster and attract people to come to your poster afterwards for further discussions.

Oral Presentation

O 01: Neuronal Activity and BDNF Drive the Expression of Nuclear Architecture Protein Satb2 in Primary Hippocampal Neurons

C. Jaitner, G. Dechant and **Galina Apostolova**

Institute for Neuroscience, Medical University Innsbruck

1st funding period

Duration: 01 March 2011 – 28 February 2013

Special AT-rich sequence binding protein (Satb2) is a transcriptional regulator that binds to AT-rich DNA sequences of multiple gene loci, alters chromatin architecture and targets chromatin remodeling/modifying complexes over long distances. We have recently shown that Satb2 plays a role in PNS neurotransmitter plasticity - it is required for the switch from noradrenergic to cholinergic neurotransmission in sympathetic neurons. In adult CNS Satb2 is expressed in pyramidal neurons of the CA1/CA2 field and in the neocortex. Interestingly, SATB2 haploinsufficiency in humans causes severe learning difficulties and profound mental retardation. To test the hypothesis that Satb2 is also important for plasticity processes in CNS we studied 1) the mechanisms of Satb2 regulation in primary hippocampal neurons and 2).

We provide evidence that both neuronal activity and brain-derived neurotrophic factor (BDNF), a key modulator of synaptic plasticity, cause an up-regulation of Satb2 mRNA and protein levels in hippocampal neurons. Satb2 induction by BDNF can be blocked by treatment with Actinomycin D, indicating that *de novo* transcription is required for the stimulation of Satb2 by BDNF. Pharmacological inhibition of mitogen-activated kinase kinase (MEK 1/2) prevents neuronal activity- and BDNF-driven Satb2 induction. Furthermore, we show that the neuronal activity-triggered Satb2 up-regulation depends on L-type voltage-gated calcium channels. Both BDNF- and neuronal activity-driven effects can be blocked by K252a, an inhibitor of Trk receptor tyrosine kinases. Taken together, our results indicate that the expression of Satb2 in hippocampal neurons is a correlate of TrkB signaling, which is known to be essential for both the induction and the maintenance of long-term plasticity processes in CNS.

To provide *in vivo* evidence supporting a role of Satb2 in CNS synaptic plasticity we are currently analyzing neuronal gene expression, synaptic function, behavioral learning and memory in conditional Satb2 knockout model.

Oral Presentation

O 02: EMT- A Possible Loop Hole for Cancer Cell Survival during Docetaxel Chemotherapy and Basis for a Highly Metastatic Cancer Cell Phenotype

Martin Puhr, Julia Hoefer, Georg Schäfer, Holger H.H. Erb, Su Jung Oh, Helmut Klocker, Isabel Heidegger, Hannes Neuwirt, and Zoran Culig

University Hospital for Urology, Innsbruck Medical University

1st funding period

Duration: 13 May 2011 – 12 May 2013

Chemotherapy with docetaxel is given to prostate cancer patients after endocrine therapy failure on the basis of prolonged survival, pain reduction, PSA response, and quality of life. However, in many cases its application is limited due to occurrence of an acquired resistance. Since Epithelial to Mesenchymal Transition (EMT) has been associated with progression of various cancers, we hypothesized that this process may be relevant for chemotherapy resistance. To uncover key mechanisms of docetaxel insensitivity we have established docetaxel resistant sublines of PC3 and DU-145 prostate cancer cells, *in vitro*.

In this study we report that docetaxel resistant cells showed a reduced expression of E-cadherin and an increased expression of the mesenchymal marker vimentin, suggesting that prolonged docetaxel treatment causes an EMT. Reduction of E-cadherin protein was mediated by decreased miR-200c/205 expression and could be reversed upon transfection of both miRNAs, which lead to an increase of apoptotic cells. Furthermore, analysis of tissue samples from patients who underwent neoadjuvant chemotherapy revealed a diminished E-cadherin and miR-200c/205 expression, suggesting a similar situation in the patients. Moreover, reduced E-cadherin expression could in addition be linked to tumor relapse.

The present study uncovers epithelial to mesenchymal transition as a hallmark of docetaxel resistance. Therefore, we suggest that this mechanism is at least in part responsible for chemotherapy failure with implications for the development of novel therapeutics.

Oral Presentation

O 03: Tetrahydrobiopterin Preconditioning Saves Murine Pancreatic Isografts from Brain Death Exacerbated Ischemia Reperfusion Injury

Rupert Oberhuber[1], Paul Ritschl[1], Cornelia Fabritius[1], Anh-Vu Nguyen[1], Martin Hermann[1], Peter Obrist[1], Ernst Werner[2], Benno Cardini[1], Manuel Maglione[1], Johann Pratschke[1] and Katja Kotsch[1]

[1]Center of Operative Medicine, Department of Visceral, Transplant and Thoracic Surgery, Innsbruck Medical University; [2]Division of Biological Chemistry, Biocenter, Innsbruck Medical University

2nd funding period

Duration: 22 July 2011 – 21 July 2013

Background: Brain death (BD) has been associated with an immunological priming of donor organs and is thought to further aggravate ischemia reperfusion injury (IRI). Recently we were able to show that the essential nitric oxide synthase co-factor tetrahydrobiopterin (BH4) prevents IRI following murine pancreas transplantation.

Herein we assessed the impact of donor BD on IRI in a murine model of syngeneic pancreas transplantation and tested the therapeutic potential of BH4 in this clinically relevant setting.

Methods: Male C57BL/6 (H-2b) mice were used as size-matched donors and recipients. Cervical heterotopic pancreas transplantation was performed using a modified no-touch technique. Animals were followed for 3h after BD induction, continuously ventilated trough a tracheostomy.

Experimental groups included (n=5 per group): non-treated BD donors (1), pre-treatment of BD donors with 50mg/kg BH4 before organ retrieval (2), ventilated non-treated donors (no BD, sham group) (3), non brain death non-treated donors (4). Following 2 hours of reperfusion, microcirculation (functional capillary density, FCD; capillary diameter, CD) as well as cell viability was assessed by intravital confocal fluorescence microscopy. Parenchymal graft damage was assessed by histology, BH4 levels were determined by HPLC and mRNA expression of inflammatory candidate markers was measured by real-time RT-PCR.

Results: BD had dramatic impact on pancreatic microcirculation 2h after reperfusion as highlighted by significantly reduced FCD as well as CD values when compared to controls non brain death ($p<0.05$). Moreover BD induced intragraft mRNA expression levels of IL-1β, TNFa, IL-6 and ICAM-1. In contrast BH4 treated pancreatic grafts showed significantly improved microcirculation after reperfusion as reflected by significantly higher FCD and CD values ($p<0.001$, respectively). BD significantly impacted cell viability 2h following

reperfusion, whereas BH4 treated grafts displayed similar percentages of viable cells in graft biopsies as non brain death controls ($p<0.001$). Early parenchymal damage in pancreatic grafts was significantly more pronounced in organs from BD donors when compared to sham or non brain death donors ($p<0.05$). Pre-treatment with BH4 however significantly ameliorated parenchymal damage in organs from BD donors ($p<0.05$).

Conclusion: This study provides in vivo evidence that donor brain death aggravates ischemia reperfusion injury after experimental pancreas transplantation. Donor pre-treatment with BH4 offers a novel therapeutic option in preventing BD exacerbated ischemia reperfusion injury.

Oral Presentation

O 04: *Galleria* Mellonella as a Host Model to study Invasive Fungal Infections due to *Mucorales*

Ulrike Binder, Elisabeth Maurer, Manuela Sparber and Cornelia Lass-Flörl

Division of Hygiene and Medical Microbiology, Innsbruck Medical University

3rd funding period

Duration: 01 August 2012 – 31 July 2014

Invasive fungal infections caused by members of the *Mucorales* (mucormycosis) have increased remarkably in the last years, with 500-1000 cases per year alone in Europe, making it the third most common invasive fungal infection after aspergillosis and candidiasis. Mucormycosis occurs mainly in immunocompromised or diabetic patients and results in unacceptably high mortality rates (50-90%), even with antifungal therapy. The great heterogeneity of the *Mucorales* is reflected in a wide antifungal susceptibility profile, differing at family, genus and species level. Many *Mucorales* exhibit an inherent resistance to most systemic antifungals, which limits antifungal therapy to the use of amphotericin B and posaconazole. A key tool to a better understanding of infections due to *Mucorales* is the use of animal models. Since the use of rodents for *in vivo* studies is very time and cost intense, alternative systems that could provide comparable data are of great advantage. The larvae of the greater wax moth, *Galleria (G.) mellonella* have been proofed to be an ideal alternative to mammals in studying *Aspergillus* and *Candida* infections. Our aim is to adapt the *Galleria* model as a quick *in vivo* screening system for antifungal susceptibility and difference in virulence of various members of the *Mucorales.* In preliminary assays we determined the suitable inoculum size for the *Mucorales*, and compared our results with those from other infection models, which correlate well. Similar to data obtained in other models, we saw differences in virulence between strains of the same species. Furthermore, we are currently analyzing the effect of amphotericin B and posaconazole treatment on the survival of *G. mellonella* infected with different members of the *Mucorales.*

Oral Presentation

O 05: The Viral Vector Vaccine VSV-GP Boosts Immune Response upon Repeated Applications

R. Tober[1], Z. Banki[1], L. Egerer[1], A. Muik[2], D. von Laer[1,], **Janine Kimpel[1]**

[1]Division of Virology, Innsbruck Medical University, Innsbruck, Austria; [2]Applied Virology and Gene Therapy Unit, Georg-Speyer-Haus, Frankfurt am Main, Germany

3rd funding period

Duration: 01 August 2012 – 31 October 2013

Vesicular stomatitis virus (VSV) is a potent candidate vaccine vector for various diseases. However, VSV's inherent neurotoxicity has limited its clinical application. Additionally, VSV induces neutralizing antibodies rapidly and is thus ineffective upon repeated applications. Our group has recently shown that VSV pseudotyped with the glycoprotein (GP) of the lymphocytic choriomeningitis virus, VSV-GP, is not neurotoxic. Here, we evaluated the potential of VSV-GP as a vaccine vector.

We used Ovalbumin (OVA) as a model antigen and analyzed immunogenicity of GP-pseudotyped and wild-type VSV expressing OVA (VSV-GP-OVA and VSV-OVA) *in vitro* and *in vivo* in mouse models.

Both vectors infected murine bone marrow-derived dendritic cells (bmDCs) *in vitro*. These bmDCs were able to activate OVA specific $CD8^+$ and $CD4^+$ T cells. Mouse experiments revealed that both VSV-OVA and VSV-GP-OVA induced functional OVA-specific CTLs and anti-OVA antibodies upon single immunization. However, boosting with the same vector was only possible for the GP-pseudotype but not for wild-type VSV. The efficacy of repeated immunization with VSV-OVA was most likely limited by the high levels of neutralizing antibodies, which we detected after the first immunization. In contrast, no neutralizing antibodies against VSV-GP were induced even after boosting. CTL responses induced by VSV-GP-OVA were as potent as those induced by an adenoviral state-of-the-art vaccine vector. Additionally, immunization with both vectors completely protected mice from infection with Listeria monocytogenes expressing OVA.

Taken together, VSV-GP is non-neurotoxic, induces potent immune responses, enables boosting and thus is a promising novel vaccine vector.

Oral Presentation

O 06: Pathophysiology of Early Brain Injury in Aneurysmal Subarachnoid Hemorrhage Patients - A Microdialysis Study

Dietmann A, Schiefecker A, Antunes AP, Pfausler B, Beer R, Sohm F, Fischer M, Lackner P, Hackl W, Thome C, Humpel C, Schmutzhard E, **Raimund Helbok**.

Department of Neurology, Innsbruck Medical University

3rd funding period

Duration: 01 August 2012 – 30 November 2013

Background: Aneurysmal subarachnoid hemorrhage (aSAH) is still associated with a high morbidity and mortality. A substantial amount of evidence from animal models indicates that early brain injury may play an important role in the patient's outcome. Underlying pathophysiologic mechanisms are still incompletely discovered in patients with aSAH and are currently not targeted as specific treatment endpoints. Cerebral microdialysis allows online measurement of brain metabolic changes and extracellular proteins including the proinflammatory cytokine interleukin-6 (IL-6) and the gelatine matrix metallopeptidase 9 (MMP-9).

Materials and Methods: Twenty-six aSAH patients with multimodal neuromonitoring were analyzed in a prospective observational cohort study. Daily cerebral microdialysates were additionally analyzed for IL-6 and MMP-9 using enzyme linked immunosorbent assays. Statistical analysis was performed using a generalized estimating equation with an autoregressive function to handle repeated observations within a subject.

Results: In the first 24 hours the patient`s metabolic CNS profile revealed brain metabolic distress and an excitatory response significantly improving over the following 5 days (*P*<0.001). Moreover we observed an increased glucose consumption reflected by a significant decrease of brain extracellular glucose concentration (*P*=0.001). Brain tissue hypoxia ($P_{bt}O_2$<20mmHg was observed in >60% of neuromonitoring time in the first 24 hours (median=15mmHg, IQR=5-22) and improved thereafter (P<0.05). Baseline IL-6 was elevated in all patients at the day of bleeding (median=4059pg/ml, IQR=1316-12456) and significantly decreased over the next 5 days (*P*<0.01). Baseline MMP-9 was initially elevated (median=851pg/ml, IQR=98-25860) and significantly decreased within 36 hours (*P*<0.05). A higher proinflammatory response was asssociated with the developement of delayed cerebral ischemia (P=0.04), whereas loss of consciousness at ictus, admisssion disease severity and early brain tissue hypoxia were associated with higher MMP-9 levels (*P*<0.03). All models were adjusted for probe location, aneurysm securing and disease severity as appropriate. Admission GCE was associated with brain metabolic distress (*P*=0.01) and a higher intracranial pressure (*P*=0.004) but not with IL-6 and MMP-9.

Conclusion: In this study we could confirm pathophysiologic mechanisms of early brain injury for the first time in patients with aneurysmal SAH, reflected by a brain extracellular proinflammatory response, MMP-9 upregulation and cerebral metabolic distress. These results need to be confirmed in a larger cohort and may be used as endpoints for future interventions targeting EBI in poor grade SAH patients.

Poster Presentation

P 01: Minor motor Phenomena during Sleep: A Quantitative Study in a Representative Sample of Healthy Sleepers

Birgit Frauscher, MD[1*], David Gabelia, MD[1], Thomas Mitterling, MD[1], Marlene Biermayer[1], Laura Ehrmann, MD[1], Deborah Bregler[1], Hanno Ulmer, PHD[2], Werner Poewe, MD[1], Birgit Högl, MD[1]

[1] Department of Neurology, Innsbruck Medical University,

[2] Institute of Medical Statistics, Informatics and Health Economics, Innsbruck Medical University

1st funding period

Duration: 22 April 2011 – 21 April 2013

Background: Many sleep disorders are characterized by increased motor activity during sleep. Differentiation between normal and abnormal motor activity is a challenge as studies on motor activity during physiological sleep are largely lacking. We quantitatively investigated a large range of motor phenomena during polysomnography in physiological sleep.

Methods: One-hundred healthy sleepers aged 19-77 years were selected from a representative population sample by a two-step screening procedure (telephone/face-to-face interviews). Polysomnography according to AASM standards was performed, and quantitative normative values were established for periodic leg movements in sleep (PLMS), high frequency leg movements (HFLM), fragmentary myoclonus (FM), neck myoclonus (NM), and REM-related EMG activity.

Results: Thirty-six subjects had a PLMS index > 5/h, 18 had a PLMS index > 15/h (90th-percentile: 24.8/h). Thirty-three subjects had high frequency leg movements (90th-percentile: 4 sequences per night). All subjects had fragmentary myoclonus (90th-percentile 143.7/h sleep). Nine subjects fulfilled AASM criteria for excessive fragmentary myoclonus. Thirty-five subjects had neck myoclonus (90th-percentile: 8.8/h REM sleep). For REM sleep, different EMG activity measures for the mentalis and flexor digitorum superficialis muscles were calculated: The 90th percentile for the SINBAR EMG activity index was 31.2%. Eight subjects exceeded the SINBAR cut-off of 32%.

Conclusion: Quantification of motor phenomena recorded during polysomnography is a basic prerequisite to develop normative values for motor phenomena during sleep and their future use in clinical routine to differentiate between normal and pathological sleep-related motor activity.

Poster Presentation

P 02: NMDA-Receptor mediated Excitotoxicity is involved in the Pathogenesis of Experimental Cerebral Malaria

Peter Lackner[1], Wechselberger KM[1], Taferner B[1], Beer R[1], Broessner G[1], Helbok R[1], Dietmann A[1], Fischer M[1], Singewald N[2]., Schmutzhard E[1]

[1]Department of Neurology, Innsbruck Medical University, [2]Department of Pharmacology and Toxicology, Center for Molecular Biosciences Innsbruck and Institute of Pharmacy, University of Innsbruck

1st funding period

Duration: 01 April 2011 – 31 March 2013

A major cause of morbidity and mortality of Plasmodium falciparum malaria is cerebral malaria (CM). The current study investigates the role of NMDA-receptor mediated excitotoxic cell death in the brain of mice with CM.

C57BL/6J mice were infected with Plasmodium berghei ANKA parasitized red blood cells. Cerebral Microdialysis was performed and glutamate levels were measured. Animals with CM were randomized for treatment with artesunate, MK801 (a non-competitive NMDA-receptor antagonist), artesunate/MK801 or vehicle. Survival and clinical outcome was scored. Brains were further processed for histochemistry.

Glutamate levels were significantly elevated in mice with CM compared to control animals. Glutamate peaks were noted before and after clinical signs of CM developed. In the treatment experiment no animal survived in the vehicle group. In contrast, 33.3% of the animals in the artesunate group and 74,1% in the artesunate/MK801 treatment group survived. Kaplan-Meier survival curves yielded a significantly longer survival of the animals in the artesunate/MK801 group compared to the vehicle or MK801 group. In addition MK801 treated animals showed significantly prolonged survival compared to vehicle treated animals. Histological analyses yielded a lower number of microhemorrhages and Fluoro-Jade B positive cells in the artesunate/MK801 treated animals compared to artesunate treated mice.

In conclusion, glutamate levels in the brain are increased early in the course of CM. Treatment with MK801, rescues mice from CM. Therefore, NMDA-receptor mediated excitotoxicity may play a role in the pathogenesis of CM and could represent a target for adjunctive treatment strategies.

Poster Presentation

P 03: Therapeutic Angiogenesis by the Neuropeptide Catestatin

Markus Theurl

University Hospital of Internal Medicine, Department of Cardiology and Angiology

1st funding period

Duration: 14 February 2011 – 13 February 2013

Introduction: Cardiovascular diseases represent the major cause of death in industrialized countries. For patients who are not eligble for revascularisation but suffering from chronic myocardial ischemia the exogenously stimulation of cardiac collateral vessels might be a promising therapeutic approach. Due to our data in the hind limb ischemia model, showing basic fibroblast growth factor (bFGF) dependent stimulation of angiogenesis, arteriogenesis and vasculogenesis by the neuropeptide catestatin (CST), we test the therapeutic potential of CST for the treatment of myocardial ischemia.

Materials/Methods: For the in-vitro experiments human coronary endothelial cells (CAEC, PromoCell) were used. To evaluate the influence of CST on coronary angiogenesis in-vitro matrigel assays were performed. CST-treatment resulted in a significant induction of capillary like tube formation comparable to bFGF, which was used as positive control. Moreover, CST mediated proliferation of coronary endothelial cells as determined by BrdU-incorporation. Interestingly, blockade of bFGF either by a bFGF-antibody or a bFGF-receptor blocker abrogated observed effects implicating involvement of bFGF in catestatin-induced effects on coronary endothelial cells. Co-incubation of CAEC with CST and a vascular endothelial growth factor-antibody didn't influence observed effects. Western blot assays revealed phosphorylation of ERK 1/2 and endothelial nitric oxide synthase by CST.

Myocardial protection from ischemia/reperfusion-injury by CST was evaluated by reversible ligation of the left anterior descending artery in C57BL/6 mice. Two injections of 10 µl of CST (each 2.5 µg) or saline (control) were performed. After one hour of ischemia and 24 hours of reperfusion mice were sacrificed and the myocardial tissue was processed for TUNEL staining. Myocytes labeled as DAPI+/TUNEL+ were considered apoptotic and counted per high power field. CST-treatment was associated with a significant reduction of myocyte apoptosis.

Discussion/Outlook: CST promotes in-vitro angiogenesis and proliferation of CAEC and protects the myocardium from ischemia/reperfusion-injury. Therefore it might be a promising peptide for the treatment of myocardial ischemia.

Poster Presentation

P 04: Treatment with Anti-IL-1β prolongs Limb Allograft Survival in an Experimental Model of Vascularized Composite Allotransplantation

Theresa Hautz

Department of Visceral, Transplant and Thoracic Surgery, Innsbruck Medical University

2nd funding period

Duration: 20 October 2011 – 19 October 2013

Background: Vascularized composite allotransplantation (VCA) serves as an excellent treatment option for reconstruction of severe tissue defects or congenital deformities. At current, however, VCA also poses a significant risk as patients are exposed to lifelong immunosuppression. Experimental studies on animals as well as the clinical experience showed that the skin is the prime target of rejection. We have previously shown that several adhesion molecules are upregulated upon skin rejection in human hand allografts. Targeting these adhesion molecules by local administration of an E- and P-selectin blocker was effective in preventing skin rejection in a rat VCA model. Cytokine expression profiles revealed that IL-1β was highly upregulated in biopsies of rejecting skin. We herein investigate the effect of an IL-1β blocker on skin rejection in a rat VCA model.

Methods: Anti-IL-1β was applied in an experimental rat hind limb transplantation model (Brown Norway (BN) rats to Lewis (LEW) rats). Four different treatment groups included: No treatment (1); baseline immunosuppression consisting of FK506 30mg/kg for 50 days and ALS 0.5mL day 0 and 3 (2), baseline immunosuppression + weekly injections of anti-IL1β subcutaneously (s.c.) into the transplanted limb (3), baseline immunosuppression + anti-IL1β s.c. into the contralateral, non-transplanted limb (4). End-point was rejection grade III or postoperative day 100. Graft infiltrating cells were isolated and assessed for CD3CD4CD25Foxp3+ T regs using flow cytometry analysis. Skin transplantations of BN and 3rd party rats were performed in long-survivors (>100 days) to assess for tolerance.

Results: Weekly s.c. injections of anti-IL-1β into the transplanted limb (group 3) resulted in significant prolongation of allograft survival (mean survival 85.3 days ± 11.2 days) as compared to the control group (2) (64±0.7 days); p=<0.05. Two group 3 animals were completely free of rejection until day 100. Weekly injections of IL-1β into the non-transplanted, contralateral hind limb (4) also prolonged allograft survival (mean survival 95.3 ± 6.6 days) compared to group 2. Animals without any treatment (1) rejected on day 7.5 ± 0.5. The proportion of graft infiltrating CD3CD4CD25Foxp3+ T regs was increased in animals receiving local anti-IL-1β (group 3) compared to other groups. BN and 3rd party skin grafts transplanted in long survivors after day 100 were rejected.

Conclusions: IL-1β is a promising target for immunosuppression in extremity transplantation. Blocking IL-1β prolongs limb graft survival when given into the allograft or the contralateral limb in a rat VCA model.

Poster Presentation

P 05: Effects of Vasopressin on Migration and Oxygen free Radical Release of Human Leukocytes

Martina Stichlberger, S Desole, K Watzinger, P Paal, W Lederer, FJ Wiedermann, CM Kaehler, M Joannidis

University Hospital for Anaesthesia and Intensive Care

2nd funding period

Duration: 15 September 2011 – 31 December 2013

Background: Vasopressin is well known for its effects on vasoconstriction, platelet aggregation and water resorption. Via the hypothalamus-pituitary axis VP takes influence on the release of corticosteroids by the adrenal glands. Many studies confirm the immunomodulatory properities of VP. It has direct pro- and anti-inflammatory effects on human leukocytes. In vitro VP induces IFN-γ-production and enhances lymphocyte response. In circulating blood cells VP binds nearly exclusively to mononuclear phagocytes. It also acts as a chemoattractant for small cell lung cancer cells. Increased VP-production seems to be an indicator for developing chronic inflammatory disease. On the other hand VP can also limit immunoresponse (e.g. in urinary tract infections by downregulating the chemokine secretion)

The aim of our study is to investigate the effects of VP on migration and chemotaxis of human leukocytes and the influence of VP on oxygen free radical release of human neutrophils in vitro. We want to get detailed information about possible benefits of VP-administration in patients with shock.

Methodology: Laboratory study. Chemotaxis experiments have just started, oxidative burst experiments will follow. Depending on the results of our preliminary investigation clinical research are invisaged. This study is a cooperation between the Department of Anaesthesiology and Critical Care Medicine and the Department of Internal Medicine Innsbruck.

Results: We hope that we can present the results of our experiments in a few weeks. Because of logistic and personal problems project start was delayed.

Poster Presentation

P 06: Impact of the Prosurvival Bcl-2 Family Member A1 on T cell Immunity

Selma Tuzlak[1], D.Tischner[1], Jan Wiegers[1] and A. Villunger[1]

[1]Division of Developmental Immunology, Innsbruck Medical University

2nd funding period

Duration: 01 August 2012 – 31 December 2013

Background: Apoptosis is a key mechanism to prevent the development of autoimmunity and cancer. It is induced either by death receptor ligation on the cell surface (extrinsic pathway) or by stress factors involving proteins of the Bcl-2 protein family (intrinsic pathway). A1/Bcl2A1 is an anti-apoptotic member of the Bcl-2 family that is mainly expressed in the hematopoietic system. Altered expression of A1 has been reported in context with autoimmune disorders as well as in different cancers in humans. In T cells A1 is thought to play a role during early T cell development and after TCR ligation upon activation. However, since no antibodies and knock-out models are available for the analysis of A1 function are available, the physiological function of A1 remains to be clarified.

Due to quadruplication in the mouse genome generation of A1 knockout mice is not feasible. Therefore, our laboratory has generated a mouse model that targets all functional A1 isoforms using an RNAi approach leading to a stable A1 knockdown in the hematopoietic system. Using this approach we are able to investigate the impact of diminished A1 expression on T cell maturation, differentiation and effector function.

Methods: Different T cell subsets were isolated from wild type mice and analyzed for A1 mRNA expression by qRT-PCR. The impact of A1 knockdown on T cell development was investigated *in vivo* by T cell subset distribution analysis, by using *in vitro* T cell development assays (OP9-DL1 differentiation system) as well as by using TCR-transgenic mice in which a mi-shRNA targeting A1 was expressed in the hematopoietic system. Furthermore, we analyzed the abundance of naïve, memory and Treg cells in the spleen and used Experimental Autoimmune Encephalomyelitis (EAE) as an *in vivo* model to study the role of A1 in T cell mediated autoimmunity.

Results: We confirmed strong A1 mRNA induction in T cells upon TCR-ligation and T cell activation. Additionally, A1-mRNA levels were found elevated in memory T cells when compared to naïve T cells. Although no impaired T cell development and T cell distribution pattern were observed in A1-knockdown mice under steady state conditions, we found a delayed onset of EAE, indicating an involvement of A1 in the development of this form of autoimmunity.

Conclusion: Diminished expression of A1 does not seem to grossly impair T cell development and T cell subset distribution under steady state conditions. This may be due to insufficient knockdown efficiency or counter-selection phenomena in our model system. However, we observed a delayed development of EAE in A1 knockdown mice compared to control mice or mice expressing a mi-shRNA targeting firefly luciferase. This strongly points towards an involvement of A1 in inflammatory responses.

Poster Presentation

P 07: The Erythropoietin-Analogue ARA290 Ameliorates the Course of Experimental Colitis

Manfred Nairz[1], T. Sonnweber[1], A. Schroll[1], E. Demetz[1], A.-M. Mitterstiller[1], I. Tancevski[1], I. Theurl[1], S.M. Mair[1], D. Haschka[1], M. Aßhoff[1], S. Berger[1], P.L. Moser[2], A. Cerami[3,4], G. Weiss[1]

[1]Department of Internal Medicine VI, Infectious Diseases, Immunology, Rheumatology, Pneumology, Medical University of Innsbruck, Austria; [2]Department of Pathology, Medical University of Innsbruck, Austria; [3]Department of Anesthesiology, Leiden University Medical Center, Leiden, the Netherlands; [4]Araim Pharmaceuticals, Ossining, New York, United States of America.

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Background: Erythropoietin (EPO) is a cytokine whose main function is to stimulate the production of red blood cells after binding of EPO to its homodimeric receptor (EPOR) on erythroid progenitors. Based on the fact that immune cells express and alternative, heterodimeric receptor composed of EPOR and CD131, we have recently reported that EPO ameliorates the course of experimental colitis due to its ability to reduce the binding activity of the transcription factor NF-κB in macrophages. The potential benefits of high-dose EPO therapy in humans are outweighed by the high risk of thromboembolic complications, though.

Methods: We used the EPO analogue ARA290, a nonapeptide known to selectively activate the heterodimeric EPOR, and tested its *in vivo* efficacy in the dextran-sulfate sodium (DSS) model of experimental colitis. Moreover, we are using cell line and primary macrophages to investigate the production of cytokines and signaling pathways involved *in vitro*.

Results: We could demonstrate that ARA290 ameliorates the clinical course of DSS-colitis as efficient as does EPO without affecting haemoglobin levels. DSS-exposed mice treated with solvent showed substantial weight loss and reduced survival. However, treatment with ARA290 or EPO resulted in significantly reduced weight loss and improved survival. Correspondingly, histopathologic analysis of colon samples revealed significantly reduced tissue damage and inflammation in DSS-exposed mice treated with ARA290 or EPO as compared to solvent-treated DSS-mice. When analyzing supernatants of colonic organ cultures for cytokine levels, we found that TNF, IL-1β, IL-6, IL-12p70, IL-23, IFN-γ and IL-17A concentrations were significantly lower following treatment with ARA290 or EPO.

Conclusion: ARA290 is efficient in improving the clinical course of DSS-induced colitis. It inhibits the production of pro-inflammatory cytokines, which are key mediators in the

pathogenesis of the disease, but does not stimulate erythropoiesis. Thus, ARA290 may be a promising agent for the therapy of humans affected by inflammatory bowel disease as it exerts potent anti-inflammatory effects without unintended thromboembolic side effects.

Poster Presentation

P 08: In Aspergillus Fumigatus, BolA-Deficiency causes Growth Defects dependent on Temperature, Oxidative Stress as well as Supply of Iron and Oxygen

Michael Blatzer, Hubertus Haas

Division of Molecular Biology/Biocenter, Innsbruck Medical University

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Background: Environmental adaptation is of paramount importance to all microbes, especially those inhabiting host niches during infection. Stress conditions encountered by pathogens within the host microenvironment are multifactorial and include changes in temperature, macro- and micro nutrient availability, oxidative stress, pH, and oxygen tension (hypoxia). Maintenance of optimal iron levels inside the cell is critical for all eukaryotes and most prokaryotes, as iron is both essential and, in excess, potentially toxic. Therefore, cells must be able to sense iron levels and maintain iron homeostasis with sufficient yet non-toxic levels of this key nutrient. Iron-sensing is best characterized in *S. cerevisiae,* where the transcription factor Aft1 senses mitochondrial Fe-S cluster biosynthesis activity involving the monothiol glutaredoxins (Grx3/4) and the BolA protein Fra2. Most other fungal species, including *Aspergillus fumigatus*, lack Aft1 homologs and control iron homeostasis using different transcription factors, homologs of *A. fumigatus* SreA and HapX. Nevertheless, most fungal species possess Fra2 homologs but information on their functions is lacking. In this study, we analysed the function of the *A. fumigatus* Fra2 homolog, termed BolA.

Materials: The function of BolA in *A. fumigatus* was studied by deletion and reconstitution of the encoding *bolA* gene with a GFP-tagged version followed by phenotyping of the strains under different growth conditions and subcellular localization.

Results: BolA-deficiency decreases siderophore production and growth during iron starvation. Remarkably, the growth defect is most pronounced at 25°C and partly cured in hypoxic conditions and raising the incubation temperature. GFP-tagged BolA localizes to the cytoplasm.

Conclusions: Growth defects caused by BolA-deficiency are affected by temperature, oxidative stress as well as supply of iron and oxygen.

Poster Presentation

P 09: Regulation of Glycolysis and Mitochondrial Respiration by BIRC5/Survivin in Neuronal Tumor Cells

Judith Hagenbuchner

Department of Pediatrics II, Medical University Innsbruck

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Adverse forms of neuroblastoma, a childhood malignancy that develops from immature neuronal progenitor cells frequently carry a gain of chromosome 17q, which leads to overexpression of the anti-apoptotic protein BIRC5/Survivin. Survivin is repressed by FOXO3, a transcription factor that is activated in response to genotoxic stress or growth factor withdrawal. We have recently shown that FOXO3 triggers biphasic ROS accumulation and subsequent apoptosis in neuronal cells. Interestingly, high endogenous levels of Survivin efficiently prevent these ROS waves and apoptotic cell death. To investigate the molecular basis of ROS- and apoptosis inhibition by Survivin the project is designed to analyze the effects of Survivin on mitochondrial architecture, mitochondrial respiration and energy metabolism in neuronal tumor cells. We already discovered that Survivin recruits the fission protein Drp1, thereby leading to reduced activity of respiration complex I and increased glycolyis. Inhibitors of glycolysis, however, lead to cell death in Survivin expressing cells. This suggests that glycolysis-inhibitors target an “archilles heel” of Survivin-overexpressing NB and may be highly useful as chemosensitizers in the treatment of high-stage NB.

Poster Presentation

P 10: Die Mitbeteiligung des Innenohrs bei der Sepsis im CLP Maus Model

Joachim Schmutzhard

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Einleitung: Hörverlust beim Intensivpatienten ist eine bekannte Komplikation. Die Sepsis als mögliche kausale Ursache wurde bis zum heutigen Tag noch nicht näher untersucht. Ziel diese Studie ist es den Einfluss der Sepsis auf das Innenohr mit Hilfe eines experimentellen Sepsis Modells bei der Maus anhand von akustisch evozierten Potentialen zu untersuchen und im Anschluss die pathologischen Veränderungen zu evaluieren.

Methode: Zur Induktion einer Peritonitis mit Sepsis wurde das CLP Model (Caecum Ligation Punktion) verwendet. Als Kontrollgruppen dienten gesunde Mäuse und SHAM Tier. SHAM Tier wurden einer Laparotomie ohne Ligatur und ohne Punktion unterzogen. Die Hörschelle wurde mittels auditorisch evozierter Hirnstammpotentialen objektiv vor Studienbeginn und präfinal evaluiert. Der Krankheitsverlauf wurde mit Vitalparametern monitorisiert. Die Innenohren wurden mittels Lichtmikroskopie, Elektronenmikroskopie und Immunhistochemie für BAX/BCL 2, Caspase 3 untersucht.

Ergebnisse: Es wurden 16 CLP Tiere, 13 SHAM Tiere und 13 gesunde Kontrolltiere in die Auswertung inkludiert. Die erfolgreiche Induktion der Sepsis konnte mit den Vitalparamenten nachgewiesen werden. Die Tiere mit Sepsis zeigten eine signifikante Hörverschlechterung im Verlauf des Experiments, während die Kontrollgruppen stabil blieben. Die Lichtmikroskopische und Elektronenmikroskopische Untersuchung erbrachten morphologische Zeichen von Apoptose in den Stützzellen des cortischen Organs. Des Weiteren konnte bei den kranken Tieren Vakuolen an der Basis der inneren Haarzellen aufgezeigt werden. Dies sind typische Veränderungen für Glutamatexitotoxizität. Die apoptotische Aktivität konnte in den Stützzellen mittels BAX/BCL 2 Verhältnis und abgespaltener Caspase 3 bestätigt werden.

Diskussion: Sepsis im Tiermodell führt zu einem signifikanten Hörverlust. Dieser lässt sich mit der Induktion von Apoptose in den Stützzellen des cortischen Organs und mit Glutamatexitotoxizität an der Basis der inneren Haarzellen erklären. Die Ursache dieser Alterationen und der Effekt bei Tieren, die die Sepsis überleben, muss an weiteren Tierexperimenten untersucht werden. Die Relevanz für den Menschen muss in weitern Studien am Sepsispatienten nachgewiesen werden.